THE EVENTS THAT LEAD TO THROMBOSIS*

EDWIN W. SALZMAN, M.D.

Department of Surgery
Harvard Medical School

Surgical Service
Beth Israel Hospital

Boston, Mass.

REGARDED in historical perspective, there are two principal hypotheses concerning the genesis of thrombosis. Each view has attracted a partisan group of adherents who have effectively championed their positions and have amassed a respectable body of evidence in the scientific literature.[1-3]

According to the more venerable and, by tradition, the more popular of these positions, thrombosis is a disease of blood coagulation, an expression of "hypercoagulability," an augmented tendency toward clotting that leads under favorable conditions to intravascular coagulation and the appearance of intravascular accumulations of fibrin.[4, 5] Because such lesions have erythrocytes enmeshed between strands of the fibrin network they appear as "red thrombi." A classical example is the peripheral venous thrombus, the bulk of whose structure resembles blood that has clotted in a test tube. Fibrin is a prominent feature of it, and there is a homogeneous distribution of erythrocytes and fibrin strands. Formation of a venous thrombus is favored by the sluggish blood flow characteristic of the peripheral veins in recumbency, by local trauma to veins, and by alterations in blood chemistry such as those in pregnancy and in the postpartum period. By permitting local accumulation of procoagulant substances, circulatory stasis allows their interaction at the site of their formation and permits the development of a fibrin clot.

Blood coagulation comes about through a series of reactions between plasma proteins that normally circulate in an inactive precursor form

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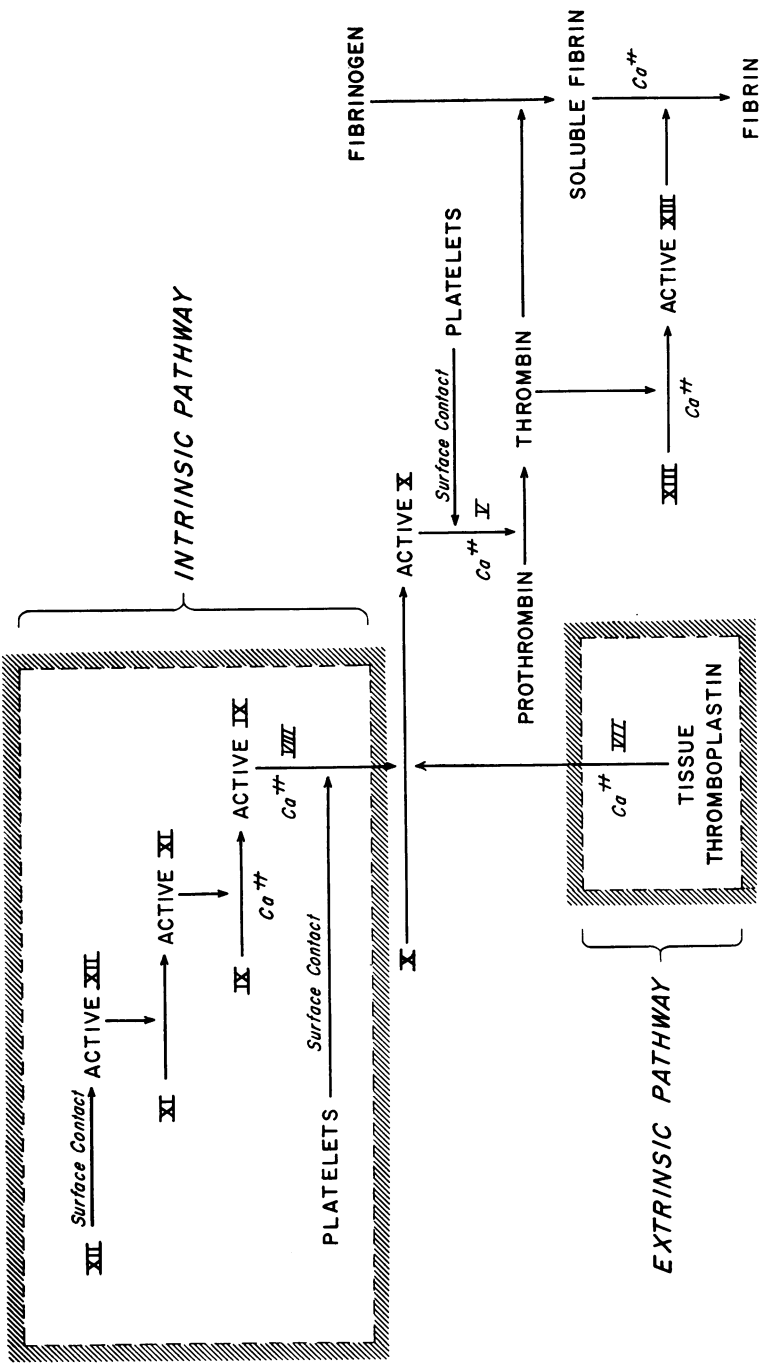

Fig. 1. Provisional scheme of blood coagulation. By convention, the coagulation factors are identified by Roman numerals. In present usage factor I is fibrinogen, II is prothrombin, III is a tissue extract involved in the extrinsic system ("tissue thromboplastin"), and IV is calcium. VI was originally assigned to an activity now considered an activated form of another factor. In the scheme represented, factors VIII and V and platelet phospholipid are thought to function as accelerators of enzymatic reactions rather than as enzymes or substrates. Activation of factor X may be achieved through interaction of plasma with lipid products of damaged tissues (extrinsic pathway) or through reactions of blood constituents alone (intrinsic pathway). Surface effects are known to be involved in initiation of the intrinsic pathway and in "activation" of platelet phospholipid (platelet factor 3) and are probably involved in initiation of the extrinsic system by particulate products of damaged tissues and in acceleration of the activation of factor X and the conversion of prothrombin to thrombin. Reproduced from Salzman, E. W.: Surface Effects in Hemostasis and Thrombosis. In: *Chemistry of Bio-Surfaces*, Hair, M. L., editor. New York, Dekker, 1971.

but are capable of conversion to proteolytic enzymes (Figure 1).[6,7] The transformation of these proteins to an enzymatically active state can be induced through pathways triggered by contact with connective tissue or with many nonbiologic surfaces ("intrinsic system") or by entrance into the circulation of lipid products of damaged tissues ("extrinsic system"). Through enzyme-substrate interaction, accelerated by combination in macromolecular complexes, protein clotting factors participate in an enzyme cascade that ultimately leads to the appearance of thrombin and thereby to the conversion of fibrinogen to fibrin.

There is evidence that blood coagulation is a continuous process in the intact circulation whose activity is offset by naturally occurring anticoagulants and by fibrinolytic activity.[8] In the experimental animal red thrombi can be produced by induction of a "hypercoagulable state" by injection of serum or other clot-promoting substances followed by interruption of the circulation, as in a segment of an intact vessel isolated between clamps.[4] As one might expect, since the formation of a red thrombus depends primarily on coagulation of plasma, it is strongly influenced by conventional anticoagulant drugs such as heparin and warfarin; common experience and carefully controlled clinical studies have confirmed the efficacy of anticoagulants in prevention and treatment of venous thromboembolism.[9]

Assessment of the blood compatibility of so-called "nonthrombogenic surfaces" by the whole blood clotting time and other coagulation tests of blood exposed to the material in question is based on the interpretation of thrombosis as an expression of disordered blood coagulation.[10] This point of view emphasizes the activation of factor XII by surface contact as the critical event in thrombosis and considers passivity toward factor XII to be the sine qua non of a surface in contact with blood. The greater the delay in induction of coagulation *in vitro*, the greater the expected compatibility of the material implanted *in vivo*.

According to an alternative hypothesis, the primary event in thrombosis is a vascular abnormality,[11-13] a disruption in the continuity of endothelium that becomes filled with adherent platelets and eventually leads to formation of a "white thrombus" (Figure 2), a plug of aggregated platelets from which erythrocytes are largely excluded and in which the contribution of fibrin is trivial. In contrast to its effect on coagulation, blood flow may accelerate the formation of a white thrombus by bringing additional platelets to the scene.[14]

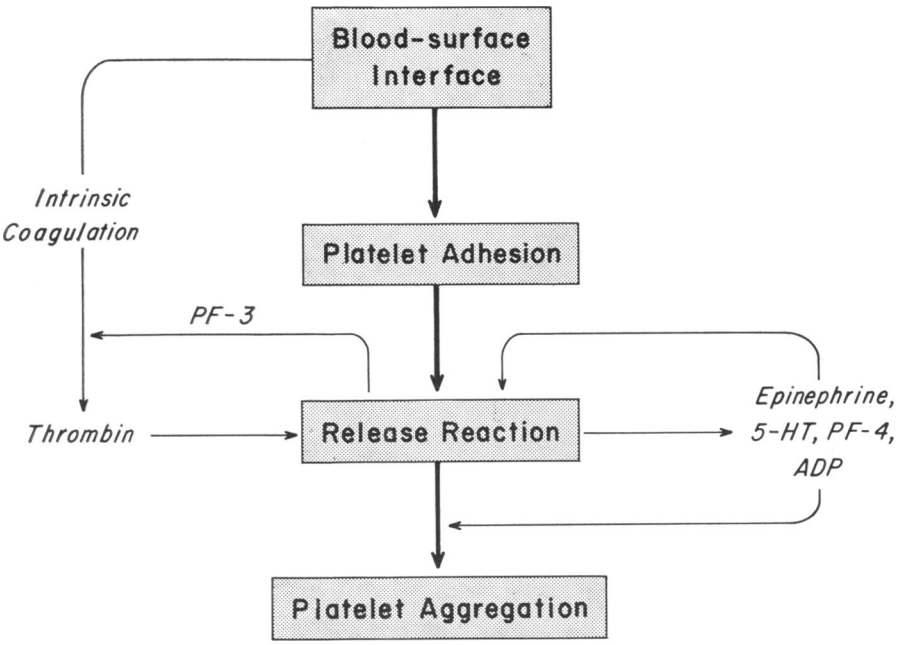

Fig. 2. Platelet-surface interaction. Adhesion of platelets leads to spreading of platelets on the substrate surface and to secretion of the contents of certain intracellular granules, the "release reaction." The materials secreted are listed in Table I. Certain of these substances can induce the release reaction in other platelets. Generation of thrombin, as a result of activation of coagulation by surface contact, also leads to the platelet-release reaction. A feature of the release reaction is the appearance of platelet-mediated clot promoting activity, known as platelet factor 3 (see Figure 1). Another effect is aggregation of platelets and formation of a white thrombus, primarily through platelet clumping induced by ADP.

Platelets do not adhere to intact endothelial cells but surprisingly minor trauma may lead to denudation of endothelium and exposure of subendothelial connective tissue composed of collagen and other materials to which platelets do adhere.[15] *In vitro*, adhesion of platelets to collagen fibers has been shown to initiate expulsion of the contents of certain platelet granules into the plasma, a secretory process known as the "release reaction" of platelets.[16, 16a] It is likely that a similar phenomenon occurs *in vivo*. Many other stimuli are known to induce the "release" of platelet constituents including contact with many nonbiologic surfaces (see Table I).[17-19] "Release" is not nonspecific disruption of the platelet. It appears to be a selective process analogous to the secretion of catecholamines by the adrenal medulla. The substances released from

TABLE I. PLATELET "RELEASE REACTION"

Inducers of release	*Substances released*
ADP	ADP
Epinephrine	Epinephrine
Collagen	ATP
Thrombin	5-hydroxytryptamine
Antigen-antibody complexes	Clot promoting activity (PF-3)
Latex particles	Antiheparin activity (PF-4)
5-hydroxytryptamine	
Surface contact	Potassium
Centrifugation	Various enzymes
Bacteria	Fibrinogen
Viruses	
Saturated fatty acids	
Trypsin	
Endotoxin	

platelet granules by appropriate stimuli include adenosine diphosphate (ADP) and adenosine triphosphate (ATP), epinephrine, histamine, serotonin, calcium, potassium, and proteolytic enzymes (Table I). Other effects include alteration of the platelet surface with the development of clot-promoting activity (platelet factor 3) and the appearance in the platelet environment of an antidote to heparin (platelet factor 4). Epinephrine, ADP, and serotonin themselves cause additional release from other platelets; the process thereby becomes self-perpetuating. An additional important effect of the release reaction is the clumping of platelets, for ADP produces alterations in the structure of platelets that lead to adhesivity, and the appearance of this nucleotide in the plasma results in the aggregation of platelets.[20] The release of constituents of platelets induced by connective tissue or, later, by the expelled platelet contents thus leads ultimately to aggregation of additional platelets and formation of a platelet plug. Thrombin also can induce the platelet release reaction,[21] and if plasma coagulation has been activated by surface-induced effects on factors XII or by entrance of lipid tissue products into the blood, the aggregation of platelets is stimulated further.

The biochemical mechanisms involved in these reactions are not entirely understood. A number of hypotheses have been advanced to

account for the aggregation of platelets by ADP, but none is entirely satisfactory.[17, 22, 23] The mechanism of the release reaction is also unclear, but an analogy is possible with secretory processes in other cells.[24] Platelets contain a contractile protein that could be involved in approximation of secretory granules to intracellular canaliculi. Fusion of their limiting membranes would permit expulsion of the granules' contents into a canalicular space in communication with the plasma. It appears that the metabolism of 3′,5′-cyclic AMP is important in these events.[25, 26] Stimuli that reduce the platelet content of cyclic AMP appear to favor platelet aggregation; elevation of platelet cyclic AMP has the reverse effect. The development of pharmacologic agents that influence the platelet metabolism of this nucleotide is a subject of interest in many laboratories at the present time.

White (platelet) thrombosis predominates in the arterial tree, where flow is rapid. The tendency of platelets to adhere to each other and to other surfaces can be expressed in quantitative terms,[27, 28] and there is evidence that the tendency of patients to develop arterial thrombi can be correlated with *in vitro* assessment of their "platelet adhesiveness."[28] Exposure of flowing blood to most artificial surfaces apparently alters platelets and leads to their removal from the circulation, either in aggregates adherent to the artificial surface or in natural filters elsewhere in the body. Expressions of this general principle are observed in the thrombocytopenia of cardiopulmonary bypass,[29, 30] in the decreased platelet survival in patients bearing artificial heart valves,[31, 32] and in the frequency of pulmonary platelet thromboemboli following extracorporeal circulation.[33]

In a platelet thrombus few of the platelets present actually adhere to the surface that initiated the process;[34] instead, for the most part, they are involved in aggregates that develop as a consequence of the release reaction. However, preliminary adhesion to a surface appears to be an essential prerequisite for the release reaction and the subsequent aggregation of platelets and the formation of a platelet thrombus. *In vitro* one can produce analogous effects in blood by addition of ADP or adrenalin, agents that produce a degree of platelet aggregation as a prelude to the release reaction and the subsequent further aggregation produced by released platelet constituents. If the system is not stirred after addition of these agents so that collisions of platelets are minimized, the release reaction does not occur.[35] Platelets swell and become spheri-

cal in the course of aggregation produced by exogenous ADP.[36] Their dramatic change in shape and volume may be analogous to the change in shape that accompanies intimate adherence of platelets to a surface. The structural alterations involved are of more than academic interest, for it is possible that such a drastic distortion of the platelet surface might itself be instrumental in the subsequent induction of the release reaction.

The concept of surface-induced thrombosis as a platelet problem has complicated assessment of potentially nonthrombogenic surfaces,[10] for *in vitro* methods of examination of platelet behavior are less straightforward and are not so well defined as are tests of coagulation. Recently much attention has been directed toward the effects on platelets of artificial surfaces, almost to the exclusion of considerations of plasma coagulation. At a recent conference which considered interaction of blood with foreign surfaces,* fewer than 10% of the papers presented dealt with plasma constituents; the remainder ignored coagulation and were devoted instead to the cellular elements of the blood.

How can one reconcile these two disparate concepts of intravascular thrombosis, the one emphasizing the interaction of plasma proteins and formation of a fibrin network, the other considering coagulation as an *in vitro* curiosity of little importance in thrombosis and concerning itself entirely with the behavior of blood cells? Surely a comprehensive approach to thrombosis must take both these concepts into account. Contact of blood with a surface other than endothelium can lead to activation of coagulation and to adhesion and aggregation of platelets, and either can predominate in the intact organism. In most circumstances, the relative contributions of plasma clotting and of platelet clumping are dictated primarily by fluid mechanics, the composition of a thrombus being largely determined by local blood flow.[14] In areas where fluid shear is minimal, as in peripheral veins or behind a stenotic mitral valve or in the area of disturbed flow with vortices and eddy currents around the sewing ring of an artificial heart valve, activation of coagulation can lead to progressively increasing concentrations of procoagulant plasma enzymes and to the development of a red fibrin clot. The more rapid flow seen in arteries or around the poppet of an artificial heart valve or in a well-engineered extracorporeal circuit does not permit

*Conference on boundary (mechanical surface, gas layer) effects on moving blood. San Diego, Calif., January 13-15, 1971. *Fed. Proc. 30*:1475-1712, 1971.

the accumulation of procoagulant dissolved plasma proteins; however, adherence of platelets to the surface and then to other platelets can occur, so a platelet thrombus can develop in such locations. Endothelial defects may lead to platelet thrombosis in the venous circulation as well as in arteries, but since the velocity of blood flow is more sluggish in veins, formation of fibrin usually predominates. In arteries the formation of fibrin cannot occur except in areas of disturbed flow such as stagnation points and regions of boundary-layer separation, and white platelet thrombi are characteristic.

To design a surface compatible with blood, one must fashion a biomaterial that exhibits sustained passivity toward plasma coagulation and platelet alteration as well, a tall order in view of our limited understanding of the features of a surface that govern either of these reactions. It is not necessarily true that passivity toward coagulation implies failure to induce platelet adhesion and aggregation, and in fact there is evidence at least in the case of heparin-coated surfaces that the two responses to surface contact are distinct. We have found that heparinized surfaces do not activate factor XII, but adsorption of platelets is a prominent feature of such surfaces exposed to native blood.[37, 38] With time in the blood stream, such surfaces change their behavior, as their adsorbed coat of plasma constituents ultimately becomes unattractive to platelets.[38] It appears that positively charged chemical groups are favorite sites for platelet adhesion, e.g., to collagen,[39] but there is convincing evidence that anions are more important in activation of factor XII, which can be inhibited by the incubation of a negatively charged surface with positively charged substances in solution.[40-42] Adhesion of platelets to a surface appears to require a protein intermediate, but since most authors report platelet function to be normal in patients congenitally deficient in factor XII, there seems little doubt that the initial phases of surface-induced coagulation are different and involve different proteins. Adsorption of platelets is favored by preliminary deposition of fibrinogen on a surface; adsorbed albumin is antagonistic to platelet adhesion.[43, 44, 37, 45] The relevance of these relations to coagulation is not certain, and the relative contribution of fibrinogen, albumin, factor XII, and other proteins to surface-blood interaction remains to be established. It is likely that the response of blood to a nonbiologic surface is chiefly determined by the composition and physical character of the adsorbed film of plasma components that initially coats the surface.[46] Analysis of the

details of this layer will undoubtedly be a major focus of attention in the next several years.

One final point needs to be made in a conference concerned with the blood compatibility of biomaterials. The fluidity of the blood can not be attributed solely to the inert nature of normal endothelium. In life, blood remains fluid not only because of the passive nonactivating lining of vessels but also because of the protective cleansing effects of flow, because of tissue filtration mechanisms that efficiently remove activated procoagulants from the circulation, perhaps because of fibrinolytic activity and because of naturally occurring anticoagulant substances in plasma. If barred from access to protective mechanisms dependent on an intact circulation, as in an isolated segment of vein or in an extracorporeal reservoir, it may be unrealistic to expect the blood to stay free of thrombus forever, even granting the eventual development of an ideally passive blood-compatible biomaterial. The blood contains within itself all the elements necessary for generation of a thrombus and, if activated to proceed, it is possible that no inert material, even one truly "nonthrombogenic," can preserve the fluidity of blood unless it is reinforced by natural defensive processes.

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